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Computer Interfaced Toxicity Testing System for Simulating Variable Effluent Loading

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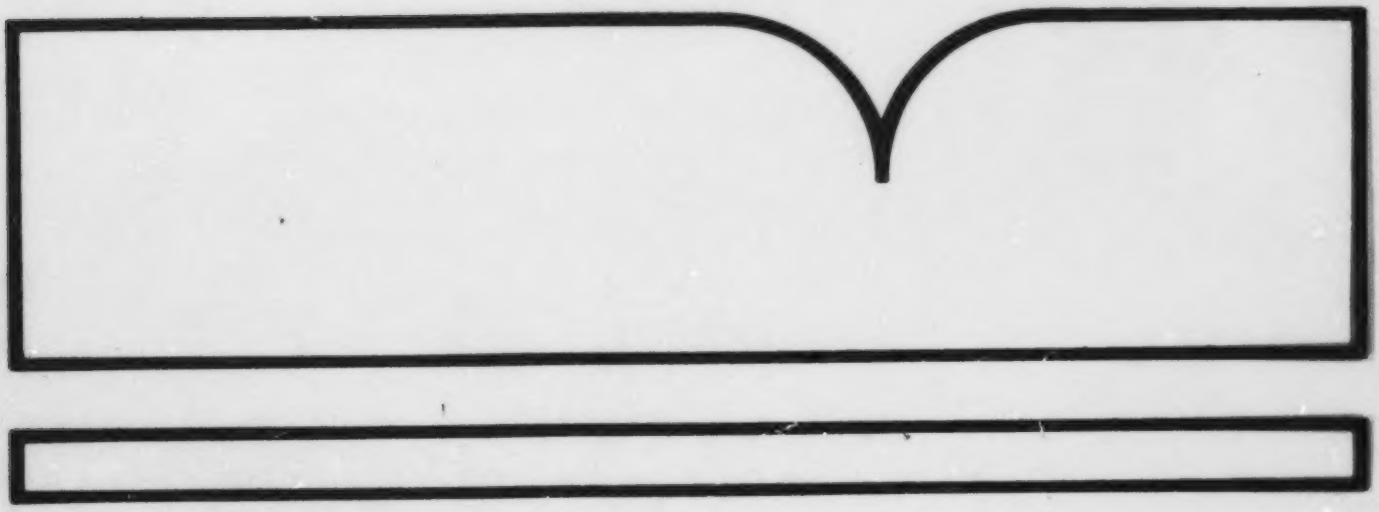

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by

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A COMPUTER INTERFACED TOXICITY TESTING SYSTEM FOR SIMULATING VARIABLE EFFLUENT LOADING

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ABSTRACT

Water quality criteria and standards are based primarily on toxicity tests carried out with single chemicals whose concentration is as nearly constant as possible. In the "real world," however, organisms are exposed to mixtures of chemicals which usually have markedly fluctuating concentrations. The primary difficulties in simulating "real world" conditions in toxicity testing are: (a) a means of varying chemical concentration to fit a predetermined set of conditions, and (b) a system which is capable of tracking and recording the response of aquatic organisms to these variations and which is quantitative and suitable for cross correlations of dose and response. Mini- and microcomputer interfacing with a toxicity testing system provides a means of systematically varying the concentration of a test chemical or chemicals in a continuous flow system. The same computer can also be used for the data acquisition system to store the voluminous time-series biological response data necessary for cross correlations with variable chemical concentrations. A description of the apparatus, examples of its use, types of data generated, and data analysis are discussed.

INTRODUCTION

Nearly all toxicity tests upon which water quality criteria are based have been carried out in test containers in which the intent was to keep the concentration of the toxicant constant. The development of continuous flow apparatus was in large part a consequence of the failure of batch test containers to maintain constant concentrations of certain kinds of toxic materials. Despite all the difficulties involved in maintaining constant concentrations and the rarity of tests in which constancy has actually been accomplished, one should bear in mind that lack of variability was the desired goal. However, when one attempts to transfer laboratory data to the "real world," one finds that constancy is the exception, not the rule, and that fluctuations in waste quality and quantity may occur from day to day, hour to hour, or even minute to minute. In spite of this lack of correlation between laboratory toxicity data and "real world" situations, toxicity tests in which the toxicant levels fluctuate according to a predetermined scheme have received little attention. This is probably due to the problems involved with automation of the toxicant controlling machinery. Consequently, applying results from relatively constant tests carried out in the laboratory to fluctuating "real world" situations presents various difficulties. Organisms are often apt to respond to thresholds rather than averages. On the other hand, duration of exposure is an

important factor in determining a response. Some thresholds, therefore, may not elicit a response if the duration of exposure is too short. Water criteria and standards (for example, National Academy of Sciences (12)) have been developed for years without substantial attention to the fluctuating exposure. Only circumstantial evidence of the effectiveness of such standards exists, and no substantial body of evidence on the relationship between fluctuating and constant concentration exposures exists for aquatic organisms. Standards developed without this information might accidentally be correct, but might just as easily be either over- or underprotective. If either of the latter situations exists, the environment may be damaged or excessive expenditures may be made for unnecessary waste treatment systems.

One basic question is: in a qualitatively fluctuating effluent discharge, when do the organisms respond to the peak concentrations as if they were constant at the peak level? Additional interesting questions are:

1. What amplitude and frequency of episodic exposures, individually and of short duration, appear to have no deleterious effects?

2. When there are sublethal responses to fluctuating concentrations, are the organisms capable of returning to normal and, if so, how much time does it take?

3. At what concentration is amplitude a more important consideration than frequency of fluctuation, and vice-versa?

4. If a large retention pond with thorough mixing and delivery of a constant concentration and quality of waste is possible and conditions permit variable releases, is a pulsed delivery of the same total amount on a daily basis more or less harmful to the exposed organisms than a constant delivery of waste?

5. Is there some way in which regulatory standards could be modified to permit occasional nontoxic pulses of short duration when large holding ponds are not available and batch processing makes pulses quite likely?

This short paper could not possibly address all these questions; however, the instrumentation making such tests feasible, one of the main obstacles to answering these questions and other related questions, can be discussed. Two major capabilities must be incorporated into the instrumentation used to solve problems: (a) a means of predetermining the amplitude and frequency of the changes in toxicant concentration, and (b) a means of recording on a regular or continuous basis the biological response to these changes so that correlations between dose and response may be achieved. A statistical analysis that will enable a quantitative evaluation of the data generated must also be developed.

The key to resolving the problem is the interfacing of minicomputers

with biological systems. This would permit the type of control necessary to make specific alterations in toxicant concentration at prescribed times, a means of handling large volumes of biological data and storing it, and a capability for pre-programming the analysis of data so that this can be accomplished in a cost-effective efficient manner.

The apparatus described herein represents an initial attempt to meet some of these requirements. This apparatus represents one of a series of uses of minicomputers in the acquisition and analysis of biological data from this laboratory (1,2,4,10,14). A first attempt to resolve a problem is usually imperfect. Nevertheless, the apparatus and the accompanying results described in this paper provide compelling evidence that sufficient promise exists for resolving some or all of the problems discussed previously and for justifying continued research and development of the apparatus. It is relatively inexpensive, simple, requires primarily shelf items, and is within the reach of most industrial facilities and nearly all major industrial research laboratories. In addition, once the apparatus is in place and functioning, it should be possible for technicians to operate and maintain it.

THEORY

The electrical action potential of muscle movement in the branchial region of a fish during ventilatory or "breathing" activity produces an electronic signal. This signal can be detected using electrodes submerged at opposite ends of a fish holding compartment (Fig. 1). When this microvolt signal is amplified to the 4 to 10 volt range, it can be recorded physically on a strip chart recorder (Fig. 2) or electronically on magnetic tape with a minicomputer.

That the ventilatory behavior of a fish will change when various toxicants, even at sublethal concentrations, are introduced into the water has often been demonstrated (6,11,14). However, the use of this principle as a continuous water quality monitor has been handicapped by the huge potential volume of data. A single unstressed bluegill (Lepomis macrochirus) can produce as many as 100,000 ventilatory signals in a 24-hour period. Natural variability requires that many individual organisms be monitored simultaneously for any analysis to be statistically valid and to prevent false indications of environmental contamination. Most workers in the past have recorded ventilatory activity intermittently with strip chart recorders and then analyzed data by personal observation, a tedious procedure at best (3,13). A more efficient use of such data is the continuous accumulation of ventilatory data from a large number of individuals over a long period of time. The most versatile and efficient method of accomplishing this is interfacing the fish with a minicomputer.

APPARATUS

An earlier version of the system now being used to investigate the ventilatory responses of the bluegill to sublethal doses of heavy metals has been described by Thompson et al. (14). Cairns et al. (5) demonstrated

that, with slight modifications, this system could be used with numerous species, both freshwater and marine.

Sensing Chambers

A fish holding chamber with a total of 16 individual 700 ml capacity monitoring compartments was constructed of plexiglas (Figs. 1 and 3). The bottom of this chamber was constructed of 6.35 mm transparent plexiglas. The ends and dividing baffles were of 3.18 mm transparent plexiglas, and the sides of each individual compartment were of 3.18 mm opaque white plexiglas. All joints were fused with methylene chloride.

The submerged electrodes were of type 304 stainless steel wire and were attached with silicone aquarium cement to the baffles at either end of the fish monitoring compartment. The electrodes were on the side of the dividing baffles opposite the fish to prevent contact between fish and electrode. Each electrode was formed in such a way that the wire never passed through the flow of water from any of the perforations in the dividing baffles (Fig. 1). The electrode wire was insulated from just below the water line, where the insulation was sealed to the wire with silicone cement to prevent water intrusion, to the barrier strip on the outside of the tank. The only exposed wire was below the water surface.

Since fish respond to visual stimuli and to vibrations in a manner similar to their response to chemical stimuli, the entire fish holding chamber was enclosed in a plywood isolation chamber. The supports of this enclosure were anchored in sand filled containers to protect against vibration (Fig. 3).

Water Delivery

Diluent was dechlorinated tap water maintained at a constant temperature of 22°C. This was delivered by gravity flow to three plexiglas mixing chambers where the diluent could be mixed with various toxicant solutions if desired (Fig. 3). The water was then passed into delivery chambers, also of plexiglas, equipped with 1 mm diameter standpipes. A constant head pressure was maintained over these standpipes by delivery in excess and the excess was drained through an adjustable standpipe. This allowed delivery of water or water and toxicant at a constant rate and concentration to each testing compartment (Fig. 3).

Data Accumulation

Each monitoring compartment was connected to an amplifier specially designed for fish ventilatory investigations (8). These amplifiers were interfaced to a PDP-8E minicomputer through a 16-channel multiplexed analog to digital converter. Data were accumulated for 15-minute periods, printed on a Decwriter II computer terminal, and stored on magnetic tape cassettes by a Decassette TU60 tape drive. These data subsequently could be transcribed by the minicomputer over a telephone line to the Virginia Polytechnic Institute and State University's main computer (an IBM/370-1580) for analyses.

Computer Program

Although it is not apparent in Figure 2, the ventilatory signal is often bimodal (7,14). This bimodality is an artifact of the amplifier design and is more pronounced in more slowly ventilating individuals (14). The signal has been visually correlated with the ventilatory activity of L. macrochirus, and each waveform, bimodal or not, represents one complete ventilatory cycle (7). The computer program developed for this system is capable of rejecting this artifact when it is present and of accumulating the total counts as well as an approximation of the average amplitude of the signal. This program also uses a software controlled variable threshold to reject low amplitude noise. Rejection of real signals is less than 2 percent, while extraneous counts are less than 1 percent.

Toxicant Delivery

An Intergral Masterflex, variable speed, peristaltic pump drive with remote control capability was used to introduce the toxicant into the mixing chambers (Fig. 3). As many as nine pump heads could be used simultaneously for delivery of different toxicant solutions to each mixing chamber or for producing various combinations, in the mixing chambers. The toxicant solutions were pumped through 1.66 mm inside diameter silicone tubing. One of the three mixing chambers was used for control and, thus, never received toxicant solution.

The minicomputer used a digital to analog converter to deliver a preselected voltage to the pump drive. A computer-pump interface was used to convert the output voltage from the computer to the optimum range for use by the pump (Fig. 4). The computer output voltage could be changed by the computer at preselected one-hour intervals to simulate fluctuating "spills" or releases. The concentration of toxicant in the individual sensing compartments reached maximum concentration in 15-20 minutes, depending on the flow rate through each compartment.

TESTING PROCEDURE

Three tests were run to test the effect of Cu^{++} on the ventilatory activity rates of the bluegill. During each test, 11 fish received applications of $CuCl_2$ solutions, and 5 were left untreated as controls. The fish were maintained under conditions of continuous light to reduce diurnal variation. The fish were not fed while in the chambers or for 24 hours before being placed in the chambers.

The fish were allowed 24 hours in the testing compartments to acclimate before ventilatory signals were recorded. Ventilatory rates were recorded then for a total of 216 hours (9 days). No toxicant was applied during the first 96 or the last 24 hours. Toxicant solutions were applied in various patterns during the intervening 96 hours. During the first test, the toxicant was delivered at a constant rate for 96 hours at a concentration of 0.5 mg Cu^{++} per liter (Fig. 5). During the second and third tests, approximately equivalent amounts were delivered in intermittent patterns. The toxicant in test two was delivered

during two variable 20-hour applications separated by a 20-hour recovery period (Fig. 6). The resulting concentrations of Cu^{++} were 0.5, 1.0, and 1.5 mg/l in 4-hour increments. Toxicant during run three was delivered in eight 5-hour applications with 1 hour increments (Fig. 7). The average concentrations during the 1-hour increments were 0.5, 1.1, and 1.3 mg/l. The variation in concentration is due to slight fluctuations in the voltage delivered to the pump by the computer.

DATA ANALYSIS

The total counts from four consecutive, 15-minute reporting periods were summed and divided by 60 to produce the average rate per minute for each 1-hour period for each individual fish being monitored. These average rates were graphed for the entire 216 hours of the test. Examples of these graphs are in Figures 5, 6, and 7.

While it is true that consecutive hourly averages for a single individual are not independent observations and that there is considerable natural variation that makes comparison between two individuals difficult, the application of a conventional one-way analysis of variance to data with large sample sizes is valid. This method was used to examine the differences between the mean rates of the combined controls and the three treatment patterns. For the purpose of the analyses the various patterns are indicated as follows. Pattern 0 = controls, all three tests combined; pattern 1 = one toxicant application; pattern 2 = two applications; and pattern 3 = eight applications. Separate analyses were performed on the pretreatment, treatment, and post-treatment data. Subsequently, a post-mortem Duncan's multiple range test was also carried out to locate possible sources of major differences.

The average rate for an entire period was calculated for each fish, and the analyses were run with these averaged data. The period for pretreatment was always 96 hours. The time for treatment was from the start of the first toxicant application until the end of the last application and varied from run to run. The time for the post-treatment period was all the remaining time after toxicant delivery ceased and was also variable.

RESULTS

Five of the 48 fish are not included in the final analysis due to various physical and electronic failures. Among the remaining 30 individuals that received toxicant in 1 of the 3 patterns, 27 showed an average increase of 16.4 counts per minute during the treatment period. The 3 that decreased did so by an average of 1.4 counts per minute. Among the 13 control fish, 8 increased by an average of 3.9 counts per minute, and 5 decreased by an average of 3.5 counts per minute.

After the toxicant flow ceased, most of the treated individuals responded with reduced ventilatory rates, and 29 of the treated fish survived the treatment period. Four of these had increased counts averaging 3.4 per minute, and 25 decreased by an average of 17.0 per minute. Among the

13 untreated controls, 3 increased by an average of 2.2 counts per minute, and 10 decreased by a 5.7 count average.

The analyses of variance indicated that there was no statistical difference between the controls and the treated individuals during either the pretreatment or the post-treatment periods; the corresponding probabilities for no difference were 30 percent and 9 percent (Table 1). Thus, the null hypothesis was acceptable at the 0.05 alpha level (Table 1). During the treatment period, the probability for no difference was 4 percent, indicating rejection of the null at the 0.05 level.

Duncan's multiple range test indicated no difference between controls and any other fish during the pretreatment period. During the treatment period, there was no detectable difference between any of the three treatment patterns. The controls, however, could not be differentiated from pattern 3, but they could be separated from patterns 1 and 2. However, it should be noted that these analyses are based on the average rate for the entire period from the start of the first toxicant application to the end of the last application, including the intermittent periods of no toxicant application. It is quite apparent from Figure 7 that there is definitely a noticable reaction in some of the individuals. That this does depend to some extent on the pattern of application can be noted from Figures 5, 6, and 7. The results of Duncan's test for the post-treatment period gave mixed results also. The treated fish from run one (pattern 1) could not be distinguished from the controls, neither could the treated fish from run two (pattern 2) or three (pattern 3) be distinguished from the controls. However, these could be differentiated from those treated with pattern 1 (Table 1).

DISCUSSION

Some tentative conclusions can be drawn from this work even though any final conclusions must await a larger data base.

1. There is a trend toward a reduction in the average rate of ventilatory activity over time. This is probably due to the fact that the fish are not fed while in the sensing chambers.

2. The presence of cupric chloride in solution at sublethal levels causes a general increase in the ventilatory activity of most bluegills which can be statistically significant if the sample size is large enough.

3. With the analyses used, differences between treated and untreated fish can be detected, but differences between application patterns cannot. However, the response to the different patterns can be distinctive as can be determined from visual inspection of the graphed data.

4. There was apparent recovery with respect to ventilatory rate in many individuals within 24 hours after

toxicant delivery ceased. However, long-term chronic effects cannot be implied from these data at present.

5. The apparatus provided the type of information it was designed to produce and merits additional attention.

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TABLE 1

Results of Analysis of Variance (ANOVA)

	Pretreatment period			Treatment period			Post-treatment period		
	F	P	df	F	P	df	F	P	df
ANOVA Results	1.26	0.30*	3,39	3.16	0.04	3,39	2.29	0.09*	3,38
Duncan's** Test Results	0 = 1 = 2 = 3			1 = 2 = 3, 0 = 3			1 = 0, 0 = 3 = 2		

* Null hypothesis may be accepted at the 0.05 α level.

** 0 = controls, 1 = continuous application, 2 = 2 applications, 3 = 8 applications.

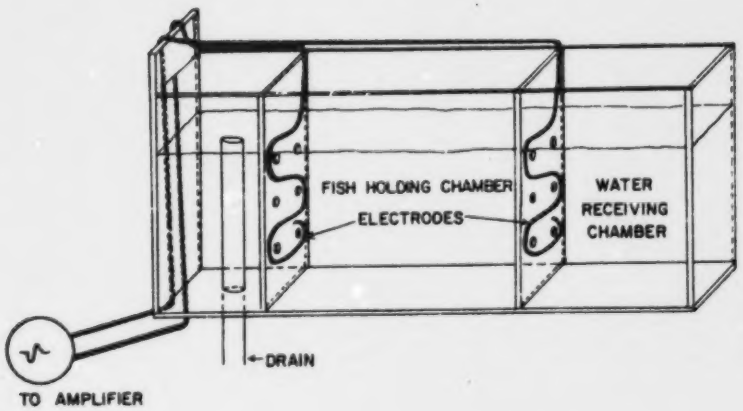

FIGURE 1 Individual monitoring compartment.

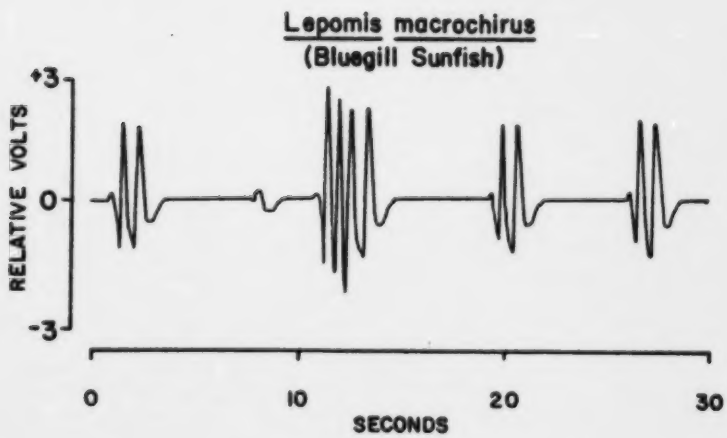

FIGURE 2 An example of the ventilatory signal as recorded on a strip chart recorder.

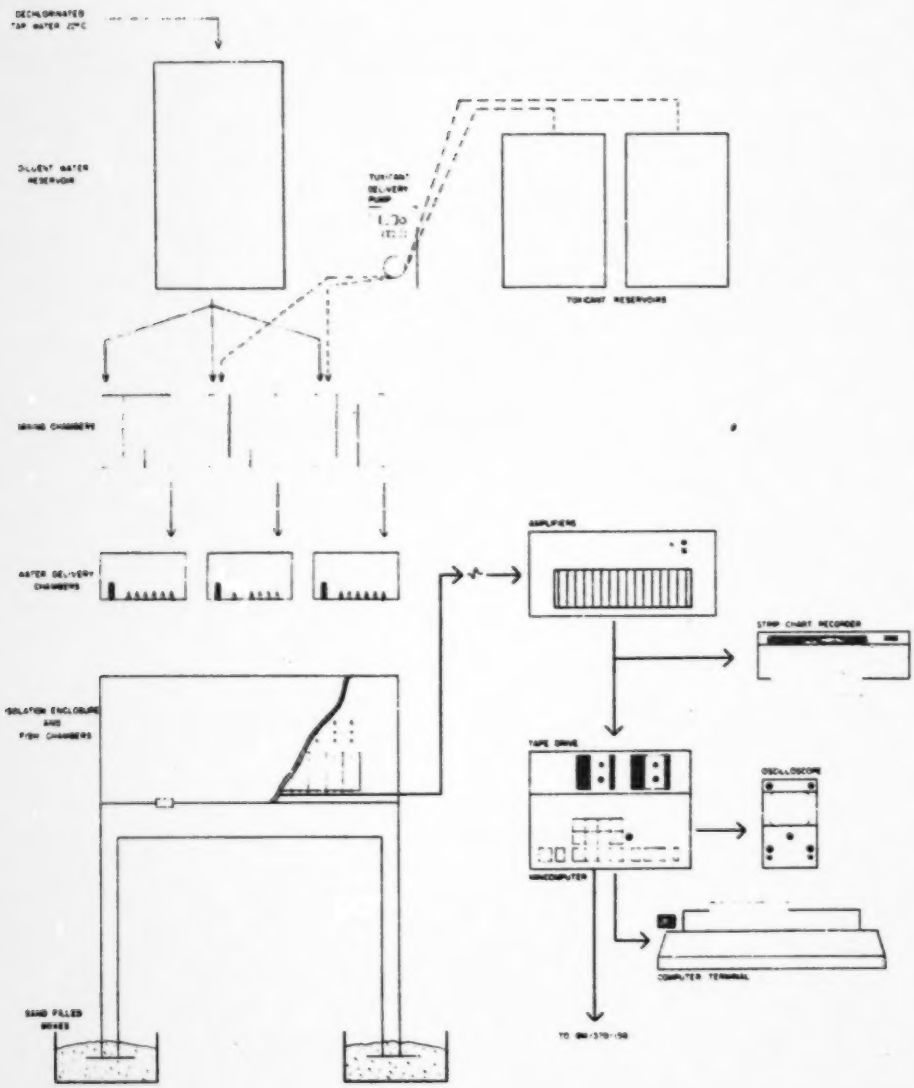

FIGURE 3 The water delivery and data accumulation system.

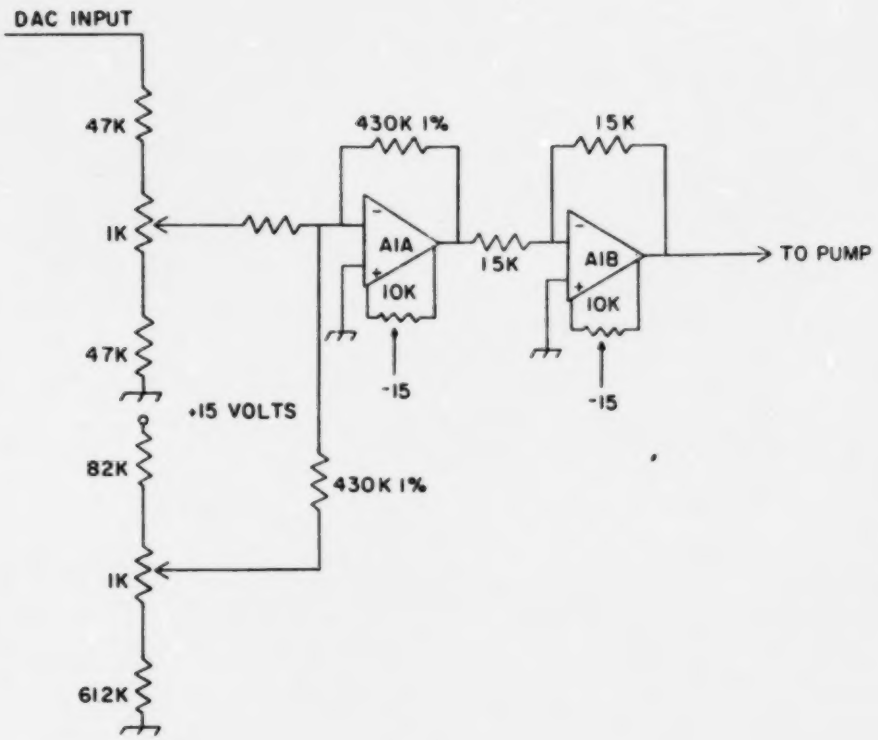

FIGURE 4 Schematic for the computer/pump interface (redrawn from Landers (9)).

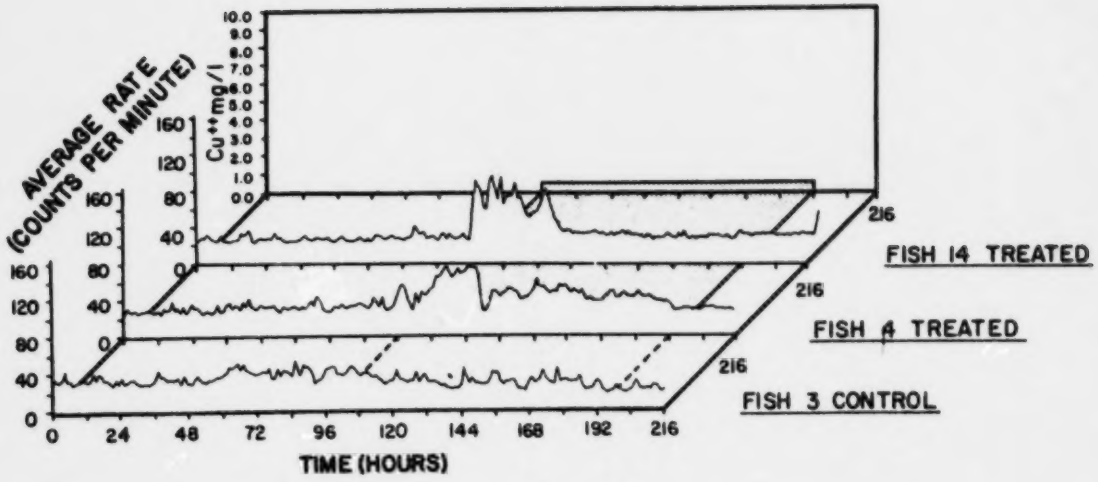

FIGURE 5 Examples of the average ventilatory rates from the first test. The individual on the first plane received no toxicant application while those on the second and third planes received 0.5 mg/l of Cu^{++} during the treatment period (shaded area). The sudden increase in ventilatory rate at the start of the treatment with a gradual reduction by the end of the treatment period was typical of most of the individuals receiving the toxicant in this pattern.

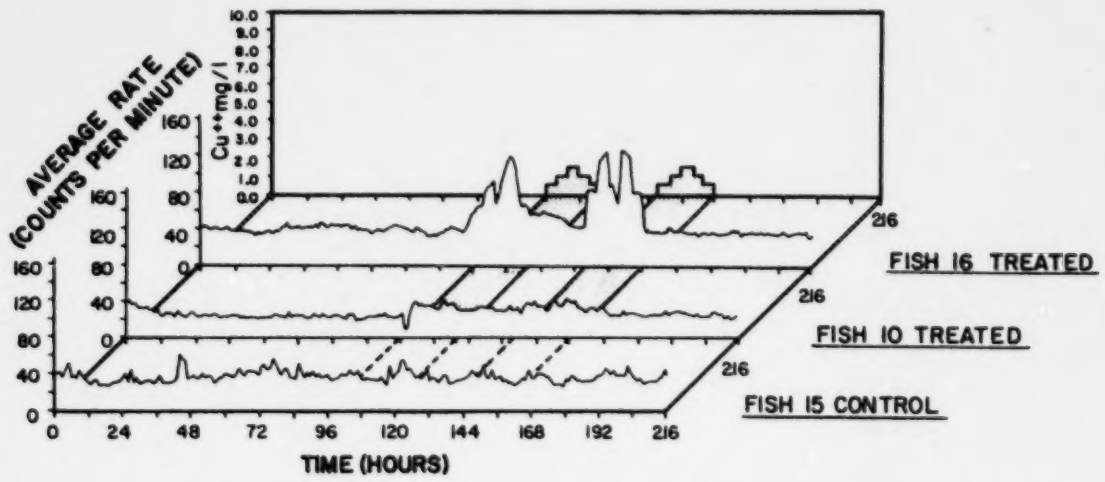

FIGURE 6 Examples of the average ventilatory rates from the second test. The treated fish received toxicant in two doses at levels of 0.5, 1.0, and 1.5 mg/l Cu^{++}. Note the variation of response among the treated fish. The two treated fish in this figure represent the extremes found among the treated individuals. The apparent reduction in rate at the peak of the second toxicant application is due to another reaction to the toxicant, a reduction in the amplitude of the ventilatory signal. While the rate probably remained in the range of 120 counts per minute, the amplitude dropped below the limit of detection. This reaction is currently being studied and will be reported in more detail at a later date.

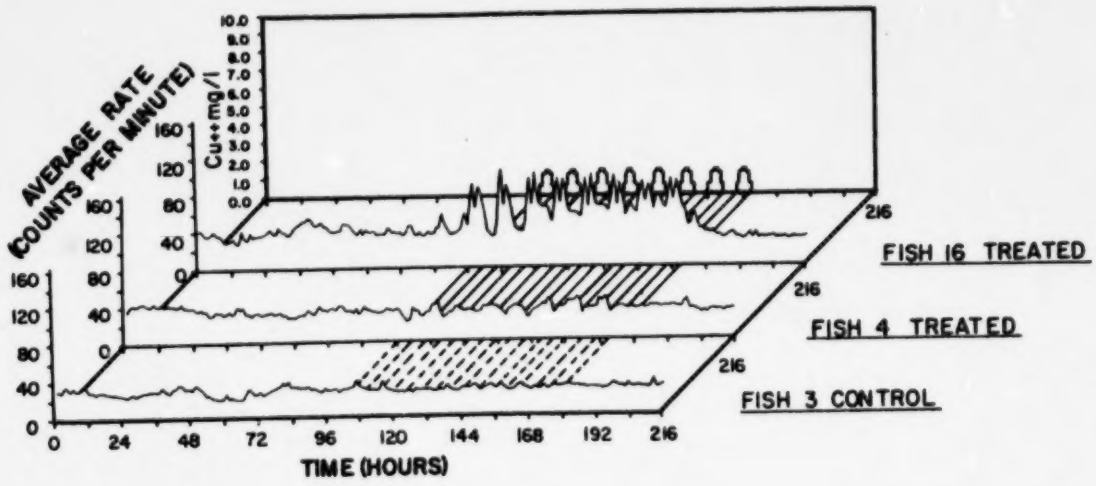

FIGURE 7 Examples of the average ventilatory rates from the third test in which the toxicant was applied in eight short bursts. The concentrations achieved were 0.5, 1.1, and 1.3 mg/l Cu^{++}. Note the variation of response among the treated fish. The two treated fish in this figure represent the extremes found among the treated individuals. The apparent reduction in rate at the peak of the second toxicant application is due to another reaction to the toxicant, a reduction in the amplitude of the ventilatory signal. While the rate probably remained in the range of 120 counts per minute, the amplitude dropped below the limit of detection. This reaction is currently being studied and will be reported in more detail at a later date.